Supplementary data for:

Cell wide responses to low oxygen exposure in *Desulfovibrio vulgaris* Hildenborough

Figure S1.

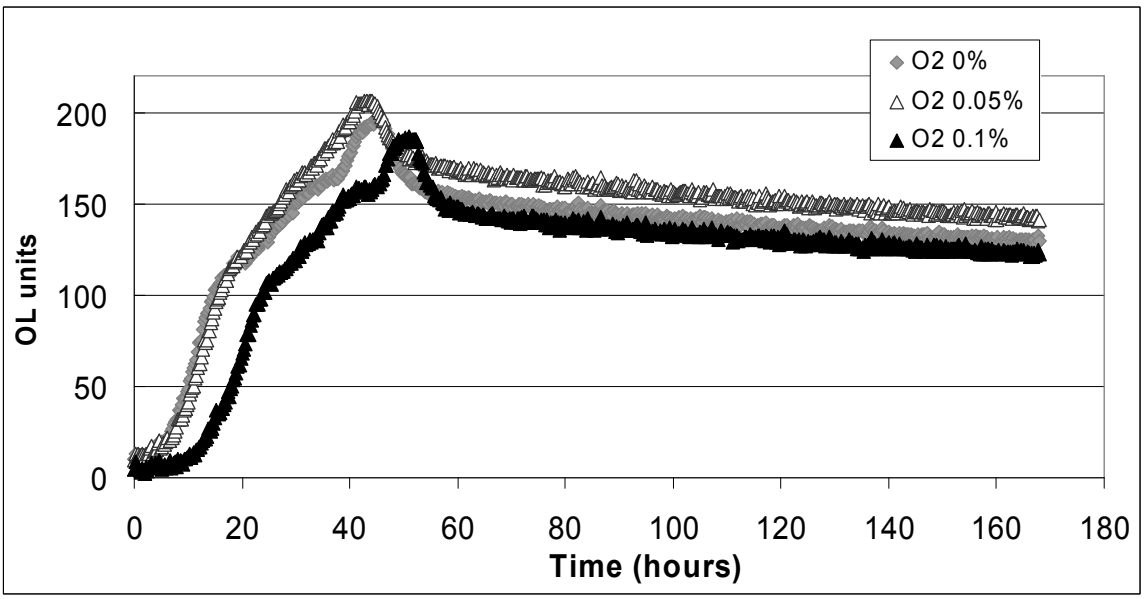


Figure S1. Recovery assay. Cells from the low O_2 exposure experiments were used to measure growth recovery. Samples were taken from the 240 min time points of the experiments shown in Figure 2 and inoculated at 10% into fresh, anaerobic LS4D medium. Growth was followed using the Omnilog over one week. Briefly, cells at mid-log phase (OD 0.35-4) were exposed to the different levels of O_2 and then transferred to a 96 well plate which was sealed in an air tight pouch. Cell growth in the plates was measured over one week using the Omnilog. The Omnilog measures growth by quantifying visual images of the plate at regular time intervals (OL units). The recovery assays indicate that the 0.1% O_2 exposure leads to a much greater lag (~15 hours) compared to the 0% and 0.05% O_2 exposed cells, prior to exponential growth.

Figure S2.

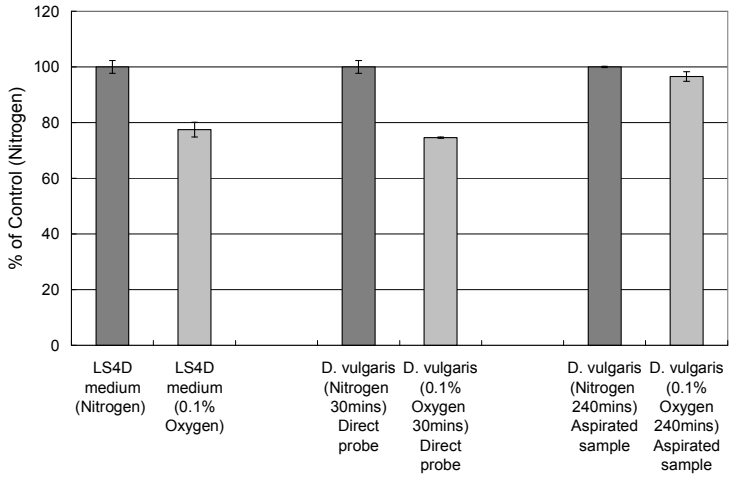


Figure S2. Measurements were taken using the Foxy oxygen measurement system (Ocean Optics, Florida) with USB2000-FL spectrometer, preconfigured for fluorescence (350-100 nm), USB-LS-450 pulsed blue LED drive module, USB-LS-450-TP10 RTD temperature probe for LS-450, and a Fospor-R oxygen sensor with silicone overcoat. Detection is reported via intensity values, which decrease as levels of O_2 increase. Therefore, decreased intensity values reflect higher levels of dissolved O_2. Both blank LS4D media and active cultures were sparged with either N_2 or 0.1% O_2 for 30 min, and the levels of dissolved oxygen were measured. The probe was allowed to equilibrate in the sample for 5 min, and then an additional 5 min of data were collected. The reported value is the average reading over the 5 minutes, with the error indicating the level of fluctuation observed during that time. The plot shows three different sets of data. For each set the control is the N_2 sparge which is set at 100%. The plot shows net difference between N_2 and 0.1% O_2 sparge. In the first set, the probe was immersed directly into bottles containing blank media as they were being sparged. A lowering in the net intensity indicates that O_2 was present in the blank media. In the second set, measurements were taken in actively growing culture. A similar net level of dissolved O_2 can be observed. In the third set, samples were aspirated from the sparge bottles into separate tubes and subsequently measured. In this case, sample handling leads to a condition where the values are no longer distinguishable.

Calculation S1:

A calculation was performed based on previously reported oxygen reduction rate (Wildschut et al J. Bact 2006) to estimate O_2 reduction by *D. vulgaris* in our experiment. The details of the calculation are shown below.

Reported specific oxygen reduction rate (ORR): $0.058\ \frac{\mu mol\ O_2}{(\min)(mg\ dry\ weight)}$

1 Absorbance Unit $OD_{600} = 0.309\ \frac{mg\ dry\ weight}{ml}$

OD_{600} at start of experiment = 0.3 AU

ORR at start of experiment:

$$(0.3)\left(0.309\frac{mg\ dry\ weight}{ml}\right)(1000\,ml)\left(0.058\ \frac{\mu mol\ O_2}{(\min)(mg\ dry\ weight)}\right) = 5.4\frac{\mu mol\ O_2}{\min}$$

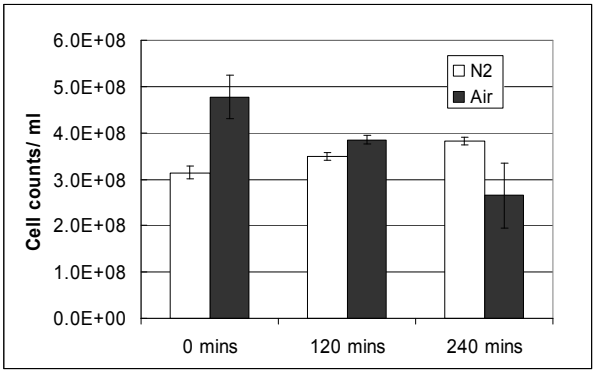
Amount of oxygen delivered:

$$\left(200\frac{ml\ gas}{\min}\right)\left(0.001\frac{ml\,O_2}{ml\ gas}\right)\left(\frac{1\,mol\,O_2}{25.4\,L}\right)\left(\frac{1\,L}{1000\,ml}\right) = 7.8\frac{\mu mol\,O_2}{\min}$$

Taken together, information from Figure S2 and the estimation above indicate that during continuous sparging of 0.1% O_2, adequate levels of O_2 are present in for a microaerobic exposure on *D. vulgaris*.

Figure S3.

A)



B)

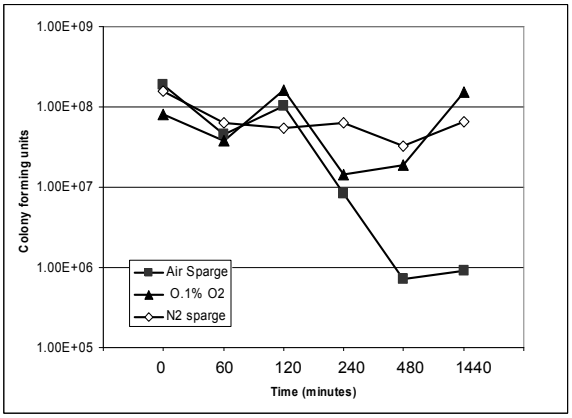


Figure S3. Cell counts and CFU measurements the different sparged cultures. The gas sparge configuration in this experiment is identical to that used in the omics experiments where cell were sparged with 100% N_2, air, or 0.1% O_2 (in N_2) as indicated in the graph. (A) Shows AODC data from cells collected at 0, 120 and 240 minutes in triplicate. (B) CFU were measured using cultures taken at different time points after initiation of sparging with N_2, air or 0.1% O_2. Aliquots were diluted serially in LS4D medium to obtain 10^2, 10^4 dilutions. Diluted cells in 200 µl were plated anaerobically in duplicate and colonies were counted after 7 days.

Figure S4.

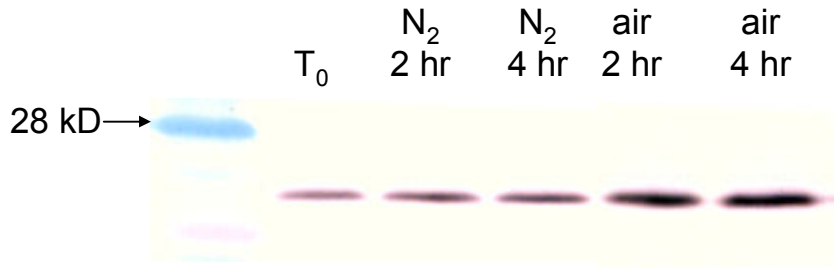


Figure S4. SOD western. The same pipeline biomass that was used for air-stressed iTRAQ proteomics is shown here. Samples were normalized by total protein (Bradford assay). Five µg total protein from each sample was used. Anti-MnSOD (Sigma) produced excellent specificity for *D. vulgaris* SOD. Only the 19-kD protein was detected. Consistent with iTRAQ quantitative data, higher levels of the SOD can be seen in the air-stressed samples.

Method: Lysate from the proteomic total protein preparation was used. After the Bradford assay, five µg total protein from each sample was separated by SDS-PAGE (4-20% Tris-Glycine, Invitrogen). Gels were transferred to nitrocellulose membrane (BioRad). Anti Superoxide dismutase, purchased from Sigma, was generated against the synthetic peptide DVWEHAYYLQYKNVRPD corresponding to the aa 183 -199 of the human mitochondrial Mn[SOD]. This sequence is also present in the Fe-SOD of *DvH*. Primary antibody was used at a 1:1000 dilution while the secondary antibody was used at 1:2000 dilution.

Figure S5:

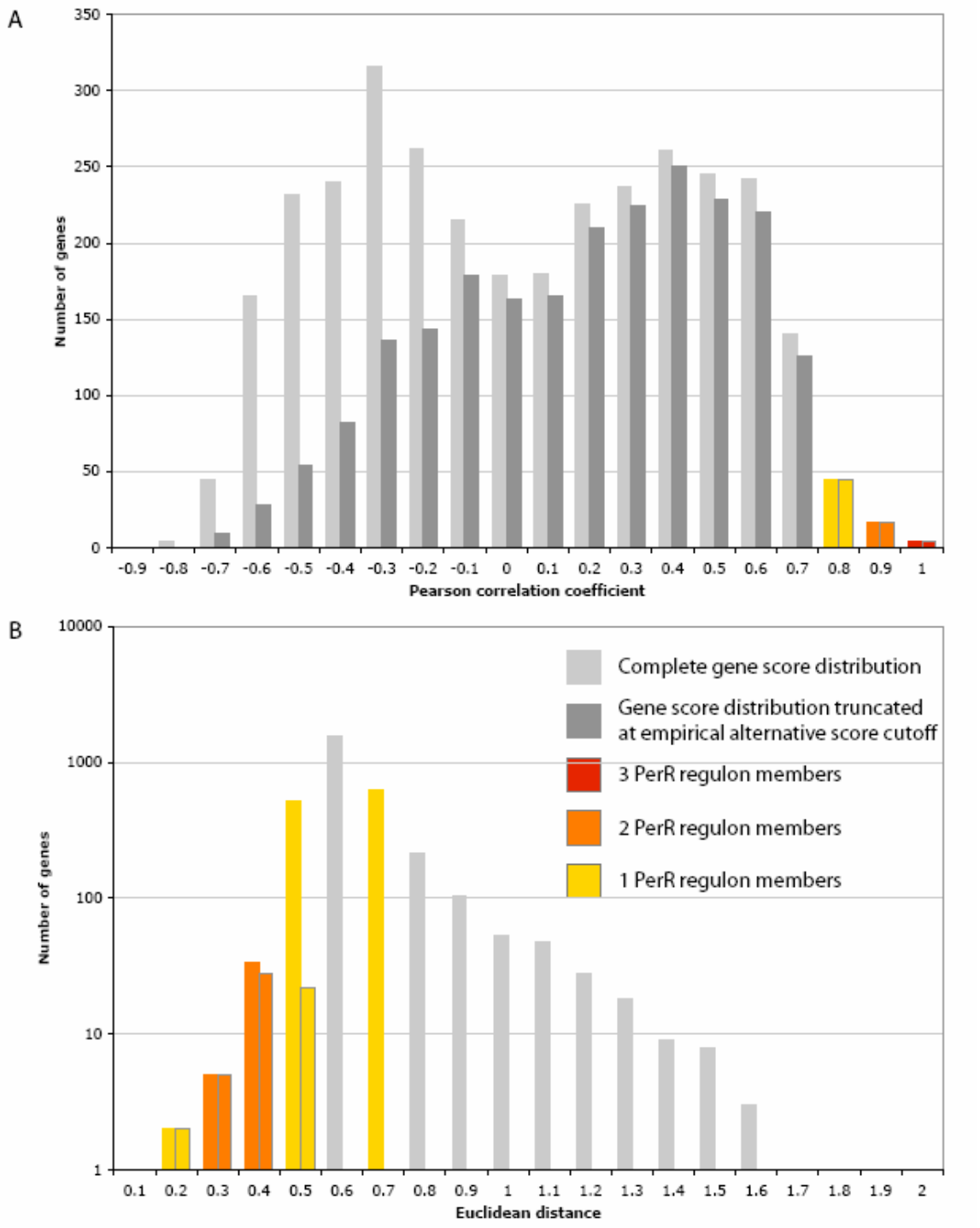


Figure S4: Histograms of two measures of gene expression similarity between *D. vulgaris* genes and the PerR regulon mean gene expression profile: Pearson correlation coefficients (A) and mean Euclidean distances (B). Filtered similarity measure

distributions are shown to highlight the relative orthogonality of the two distance measures and the stronger effect of the Pearson correlation cutoff. The filtering relies on applying empirical similarity measure cutoffs based on one measure to distributions ranked using a different similarity measure. In order to capture information on the direction of gene expression change we first applied a Pearson correlation cutoff, the stricter of the measures, and subsequently sorted by the mean Euclidean distance to better account for the magnitude of change and effects of missing data. For the Pearson correlation (A), the truncated distribution (dark gray bar or bar border) corresponds to discarding values ≥ the largest Euclidean distance of any member from the PerR regulon to the mean PerR regulon gene expression profile. For the Euclidean distance (B), the truncated distribution corresponds to discarding values ≤ the smallest Pearson correlation of any member of the PerR regulon to the mean PerR regulon gene expression profile. Histogram bins containing PerR regulon members are colored by the number of sequences from the regulon found in that bin. All sequences from the PerR regulon fall into bins corresponding to highest similarity, with a more scattered distribution for Euclidean distances. The truncated similarity distribution as in (B) corresponds to the data represented in the heatmap of the PerR profile search results (Figure 9).

It should be noted that due to the limited number of experimental time points, the standard Pearson correlation coefficient statistical significance test using the t-distribution was not meaningful for the profile search results. The limited data also implies only a small number of possible expression pattern outcomes and thus random matches may be expected by chance. We performed simulations to determine the significance of matches to expression profiles by sampling 10,000 sets of six genes (modeling the size of the PerR regulon, requiring complete microarray data across all time points), computing a mean gene expression profile, and performing a profile search against the microarray data in this study. We compared results of this simulation with profile searches based on mean gene expression profiles of 378 *D. vulgaris* operons (requiring complete microarray data across all time points). This analysis revealed enrichment over random in the operon profile search results only for genes with the most highly correlated expression profiles (Pearson correlation ≥ 0.95) (data available upon

request). Additionally, we relied on biological validation using operon structure and functional annotations to assign significance to the profile search results.

All microarray data can be found using the link:

http://www.microbesonline.org/cgi-bin/microarray/viewExp.cgi?locusId=&expId=28+74

where 1000ppm corresponds to 0.1% O_2 and Air corresponds to air.

The links for raw data are:

0.1% O_2: http://www.microbesonline.org/microarray/rawdata/exp28_E35

air: http://www.microbesonline.org/microarray/rawdata/exp74_E12

0.05% O_2: http://www.microbesonline.org/microarray/rawdata/exp16_E29